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Research Article

Systematics of *Parnassia* (Celastraceae) inferred from plastid and nuclear DNA sequence data

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Abstract

We presented a comprehensive phylogenetic analysis of *Parnassia* covering approximately 80% of recognized species. By integrating five plastid markers (*rbcL*, *matK*, *rpl32-trnL*, *trnT-trnL* and *trnL-trnF*) with nuclear marker ITS sequences and applying both maximum parsimony and Bayesian inference approaches, we reconstructed robust phylogenetic relationships within the genus. We presented notable differences between molecular phylogeny and the established sectional classification. While *Parnassia* sect. *Fimbripetalum* emerged as monophyletic with strong support, *P.* sect. *Saxifragastrum*, *P.* sect. *Cladoparnassis*, *P.* sect. *Nectaroquinquelobos*, and *P.* sect. *Allolobos* were resolved as paraphyletic. The monotypic *P.* sect. *Nectarobilobos* represented a distinct evolutionary lineage, whereas the species-rich *P.* sect. *Nectarotrilobos* was dispersed across multiple clades. Notably, three eastern Himalayan species (*P. faberi*, *P. esquirolii* and *P. labiata*) formed a well-defined clade with highly reduced staminodia. This study provided a critical molecular framework for reevaluating morphological evolution and revising the infrageneric classification of *Parnassia*.

Key words: Character evolution, Chloroplast DNA, ITS, Molecular phylogeny, *Parnassia*

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Introduction

The genus *Parnassia* L. (Celastraceae) comprises 50–70 species of perennial herbs predominantly distributed across arctic and temperate regions of the Northern Hemisphere (Phillips 1982; Hultgård 1987; Ku 1987; Wu et al. 2003; Simmons 2004; Wu 2005; Shu 2017). The genus is readily identified by its basal leaf rosettes, elongated peduncles bearing solitary flowers, pentamerous floral organization, and the distinctive presence of staminodia opposite each petal (Nekrassova 1917; Handel-Mazzetti 1941; Phillips 1982; Ku 1987; Gu and Hultgård 2001; Simmons 2004; Shu and Zhang 2017). Mountains of Southwest China and the Himalaya harbor centers of diversity, with more than 30 endemic species (Hultgård 1987; Wu et al. 2003; Shu et al. 2017; Dai et al. 2021, 2023). In contrast, North America possesses only approximately 10 species, including

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the widespread circumpolar *Parnassia palustris* and the Siberian-North American *Parnassia kotzebuei* (Hultgård 1987; Bonnin et al. 2002; Borgen and Hultgård 2003; Weber 2003).

Since its establishment by Linnaeus in 1753, the systematic position of *Parnassia* has been debated and historically placed in various families, notably Saxifragaceae *sensu lato* (Hooker and Thomson 1858; Dandy 1927; Engler 1930, 1964; Thorne 1976; Bohm et al. 1986; Ku 1995), with other proposed affinities. Contemporary molecular systematics, however, firmly supports its inclusion within Celastraceae (APG IV 2016).

Within this established familial framework, molecular phylogenetic studies consistently identify the monotypic genus *Lepuropetalon* Elliott as sister to *Parnassia* (Simmons et al. 2001; Simmons 2004; Zhang and Simmons 2006; Yang et al. 2012), a relationship corroborated by both vegetative and floral morphology (e.g., Murbeck 1918; Spongberg 1972; Gornall and Al-Shammary 1998; Simmons 2004). Broad-scale molecular analyses consistently resolve the [*Lepuropetalon* + *Parnassia*] clade as sister to Celastraceae (e.g., Soltis and Soltis 1997, 2000; Soltis et al. 2011), with more focused family-level studies positioning this clade within an early-diverging Celastraceae lineage with weak (Simmons et al. 2001; Bacon et al. 2016) to high statistical support (Zhang and Simmons 2006; Xia et al. 2022).

Infrageneric classification presents equally complex challenges. Drude (1875) established an infrageneric taxonomic system of *Parnassia* and recognized four sections: *P.* sect. *Nectarotrilobos*, *P.* sect. *Fimbripetalum*, *P.* sect. *Saxifragastrum*, and *P.* sect. *Nectarodroson*, which was later expanded by Engler (1930) to include *P.* sect. *Cladoparnassia*. While most species of *P.* sect. *Nectarodroson* are from North America with the exception of *P. palustris* and *P. kotzebuei*, species of other sections in the genus are concentrated in the Altai and Himalayan regions of Asia. Asian taxa exhibit significant morphological diversity (Handel-Mazzetti 1941; Gu and Hultgård 2001), with species identification relying heavily on staminodial characteristics, petal margins, and cauline leaf number (Fig. 1). Phillips (1982) subsequently divided Drude's *P.* sect. *Nectarodroson* (≡ *P.* sect. *Parnassia*) into two smaller sections (*P.* sect. *Parnassia* and *P.* sect. *Longiloba*). Built on the taxonomic framework of Drude (1875), Ku's (1987) treatment of Chinese species added four additional sections, viz. *P.* sect. *Odontohymen*, *P.* sect. *Nectarobilobos*, *P.* sect. *Allolobos*, and *P.* sect. *Nectaroquinquelobos*, based primarily on the staminodium traits, petal entire or fimbriate, and number of cauline leaf. Wu et al. (2003) proposed a modified classification system based on Ku's (1987) framework. Thus, current infrageneric taxonomy primarily reflects the systems of Drude and Engler's (1930) in Table 1.

Molecular systematics has rapidly advanced our understanding of *Parnassia* phylogeny. Zhang and Simmons (2006) confirmed the monophyly of the genus but with limited intrageneric resolution due to sparse taxon sampling. Our previous work (Yang et al. 2012) enhanced geographical representation and explored DNA barcoding applications. Xia et al. (2022) provided insights into chloroplast genomics and codon usage analysis in *Parnassia*. Nevertheless, a comprehensive phylogenetic framework incorporating North American representatives remains lacking. Here, we employ an expanded dataset comprising

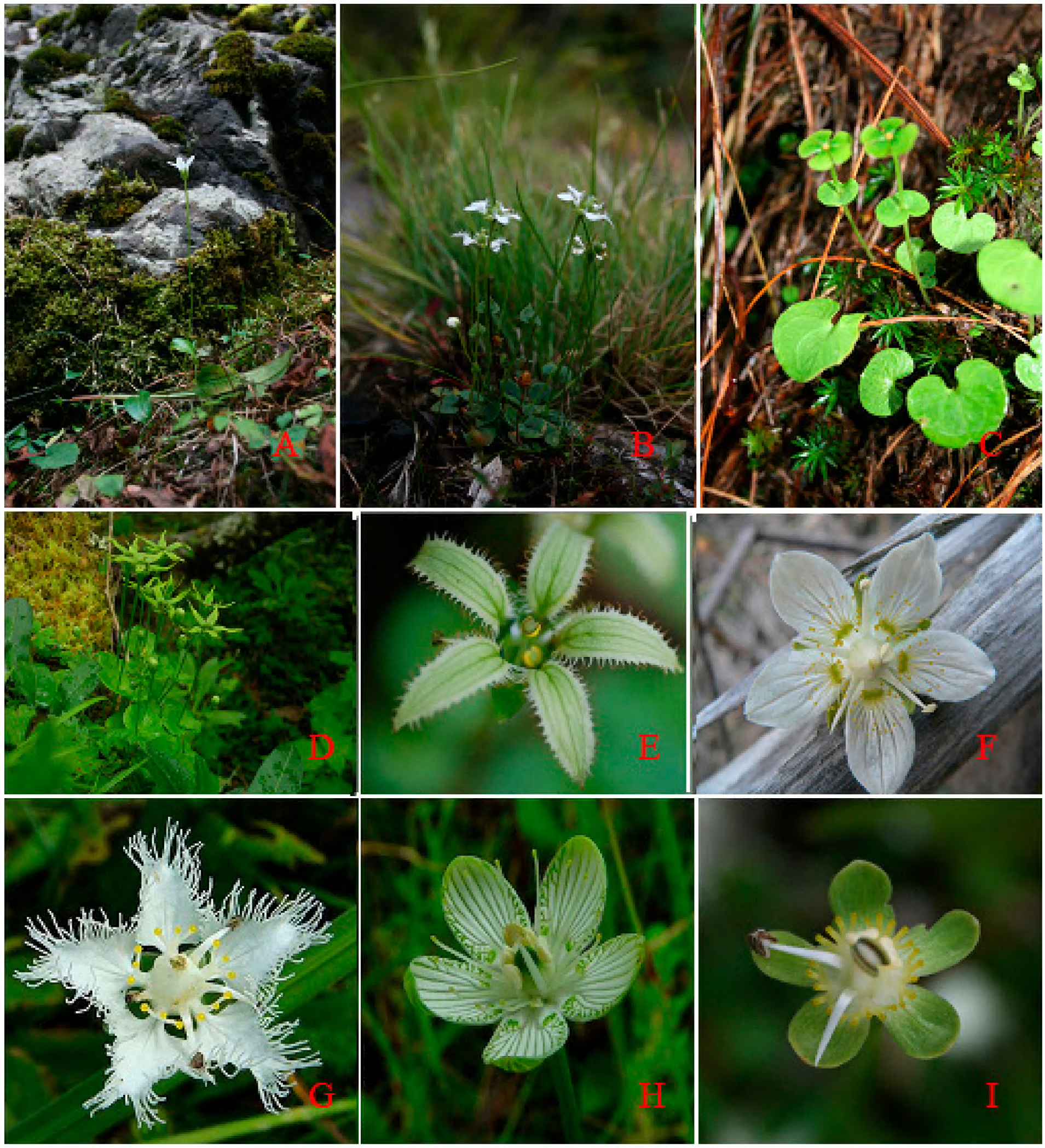

Figure 1. Habitat and petal and staminodial characteristics of *Parnassia*. **A–D.** Natural habitats: **A.** *P. laxmanni* (*P.* sect. *Nectarotrilobos*); **B.** *P. farreri* (*P.* sect. *Odontohymen*); **C.** *P. tenella* (*P.* sect. *Saxifragastrum*); **D.** *P. longipetala* (*P.* sect. *Saxifragastrum*); **E–I**, Petal margins and staminodial morphology: **E.** *P. cooperi* (*P.* sect. *Nectarotrilobos*); **F.** *P. palustris* (*P.* sect. *Parnassia*); **G.** *P. foliosa* (*P.* sect. *Fimbripetalum*); **H.** *P. grandifolia* (*P.* sect. *Parnassia*); **I.** *P. wightiana* (*P.* sect. *Allolobos*). Photos by Ding Wu and Shu- Dong Zhang.

five cpDNA regions (*rbcL*, *matK*, *rpl32-trnL*, *trnT-trnL*, *trnL-trnF*) and nrDNA ITS. Increased taxon and character sampling for *Parnassia* is expected to (1) reconstruct a robust molecular phylogeny of *Parnassia*; (2) critically evaluate existing infrageneric classifications; (3) identify morphological synapomorphies for major clades; and (4) establish a phylogenetically-informed taxonomic framework.

Table 1. Comparative overview major infrageneric classifications in *Parnassia* (number of species in parentheses).

Drude (1875) & Engler (1930)	Ku (1987)	Wu et al. (2003)
Sect. *Parnassia* (13)	Sect. *Parnassia* (1)	Sect. *Parnassia* (1)
Sect. *Fimbripetalum* (6)	Sect. *Cladoparnassia* (2)	Sect. *Allolobos* (10)
Sect. *Nectarotrilobos* (23)	Sect. *Fimbripetalum* (4)	Sect. *Fimbripetalum* (4)
Sect. *Saxifragastrum* (9)	Sect. *Nectarobilobos* (1)	Sect. *Nectarobilobos* (1)
Sect. *Cladoparnassia* (1)	Sect. *Nectaroquinquelobos* (4)	Sect. *Xiphosandra* (3)
	Sect. *Nectarotrilobos* (30)	Sect. *Odontohymen* (2)
	Subsect. *Amblysandra* (27)	Sect. *Nectarotrilobos* (28)
	Subsect. *Xiphosandra* (3)	Sect. *Cladoparnassia* (3)
	Sect. *Odontohymen* (2)	Sect. *Saxifragastrum* (8)
	Sect. *Saxifragastrum* (10)	
	Sect. *Allolobos* (5)	

Materials and methods

Taxon sampling

Our sampling strategy encompassed 45 *Parnassia* species, representing approximately 80% of the genus and all sections recognized by Ku (1987). Most material was collected from wild populations in China, supplemented by herbarium specimens (K, E, US, BH). Based on strong evidence supporting *Lepuropetalon* as sister to *Parnassia* (e.g., Simmons et al. 2001; Zhang and Simmons 2006; Soltis et al. 2011) and indications of relationship to *Mortonia*, *Microtropis*, *Pottingeria*, *Quetzalia*, and *Zinowiewia* (Zhang and Simmons 2006; Simmons et al. 2012), we selected five species from these genera as outgroup taxa. Voucher details and GenBank accessions are presented in Suppl. material 1.

Molecular marker amplifications

We extracted total DNA from silica gel-dried leaves and herbarium specimens using a modified CTAB protocol (Doyle and Doyle 1987). DNA quality was verified by 1.5% agarose gel electrophoresis. We amplified and sequenced the ITS region with primers ITS5/ITS4 (White et al. 1990), the *trnL-trnF* region (including the *trnL* intron) with primers c/f (Taberlet et al. 1991), the *trnT-trnL* intergenic spacer with primers a/b (Taberlet et al. 1991), and the *rbcL* gene with primers *rbcL* a-f (Kress and Erickson 2007)/724R (Fay et al. 1997). The *matK* gene was amplified using Xf/5r primers (Ford et al. 2009).

Polymerase chain reaction (PCR) amplifications were conducted in a 20 µL reaction mixture containing 1 × *Taq* buffer [50 mM $(NH_4)_2SO_4$; 75 mM Tris-HCl (pH 8.3); 50 mM KCl; 0.001% gelatin]; 2.5 mM $MgCl_2$, 0.4 mM of dNTPs, 0.5 µM of each primer, 1.0 U of *Taq* DNA Polymerase (TaKaRa Biotechnology Co. Ltd., Dalian, China), and 1 µL of genomic DNA (25–30 ng). Thermal cycling conditions comprised: an initial template denaturation at 94 °C for 5 min, 35 cycles of 30 seconds denaturation at 94 °C, 1 minute primer annealing at 52 °C, 1.5 min extension at 72 °C, with a final extension of 8 min at 72 °C. Amplification products were purified using the QIAquick Gel Extraction Kit. Purified PCR products were sequenced bidirectionally using the PCR primers on an ABI 3730 DNA Sequencer (Applied Biosystems, Inc.).

Phylogenetic analyses

Sequence alignments were performed using MAFFT v7.409 (Katoh and Standley 2013) and manually refined in BioEdit Sequence Alignment Editor v.7.0.0. We assessed dataset congruence using the incongruence length difference (ILD) test in PAUP*4.0b10 (Swofford 2002) following Wang et al. (2004). Based on the ILD test results, we analyzed three datasets separately: nrDNA ITS, cpDNA regions (*rbcL*, *matK*, *rpl32-trnL*, *trnT-trnL*, *trnL-trnF*), and a combined 5-gene dataset. Phylogenetic reconstruction employed maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) approaches.

We determined the optimal substitution model (GTR+G+Γ) using ModelFinder v2.1.6 (Kalyaanamoorthy 2017) under the Akaike information criterion (AIC). ML analyses were conducted with RAxML 8.2.X (Stamatakis 2014) using 1,000 rapid bootstrap replicates. BI analyses were performed with MrBayes v 3.2.7a (Ronquist et al. 2012) via the CIPRES Science Gateway V 3.3, nodes with posterior probabilities (PP) > 0.95 were considered significant.

Results

Sequence characteristics

The ILD test detected no significant incongruence among the five cpDNA regions (P > 0.01). The aligned ITS region spanned 738 bp (including adjacent regions of the 18S and 26S genes sequences), with the 5.8S subunit exhibiting high conservation (165 bp). The combined chloroplast dataset (4049 bp) contained 787 variable sites (19.4%), including 568 parsimony-informative characters (14.0%). The *trnT-trnL* (605 bp) and *matK* (861 bp) markers showed the highest variability (227 and 254 variable sites, and 190 and 170 parsimony-informative sites, respectively), while *rbcL* (711 bp) and *rpl32-trnL* (836 bp) were more conserved (75 and 126 variable sites, 50 and 78 parsimony-informative characters, respectively). The *trnL-trnF* intergenic spacer exhibited complete congruence with *trnT-trnL*, with all 105 variable sites being parsimony-informative characters.

Plastid phylogeny

Combined cpDNA analyses strongly supported the monophyly of *Parnassia* (BS=100, PP=1.0), resolving six major clades (Fig. 2). Clade I (BS=83, PP=1.0) comprised three Chinese species (*P. faberi*, *P. esquirolii*, *P. labiata*). Clade II contained only *P. bifolia*, positioned basal to a large clade encompassing clades III-VI. Clade III (BS=100, PP=1.0) included *P. lutea* and *P. scaposa*. Clade IV (BS=85, PP=0.99) united part of *P.* sect. *Nectarotrilobos* characterized by ovate-oblong basal leaves and three- lobed staminodia. Clade V (BS=98, PP=1.0) contained primarily North American species divided into two subclades (A: BS=100, PP=1.0; B: BS=100, PP=1.0), though *P. kotzebuei* extends into Siberia and *P. palustris* exhibits circumboreal distribution.

Clade VI (BS=100, PP=1.0), the largest and probably the most recently diversified, included nearly half of all sampled species, predominantly from the Himalayan- Hengduan Mountains (except *P. procul* in North Sumatra). Within

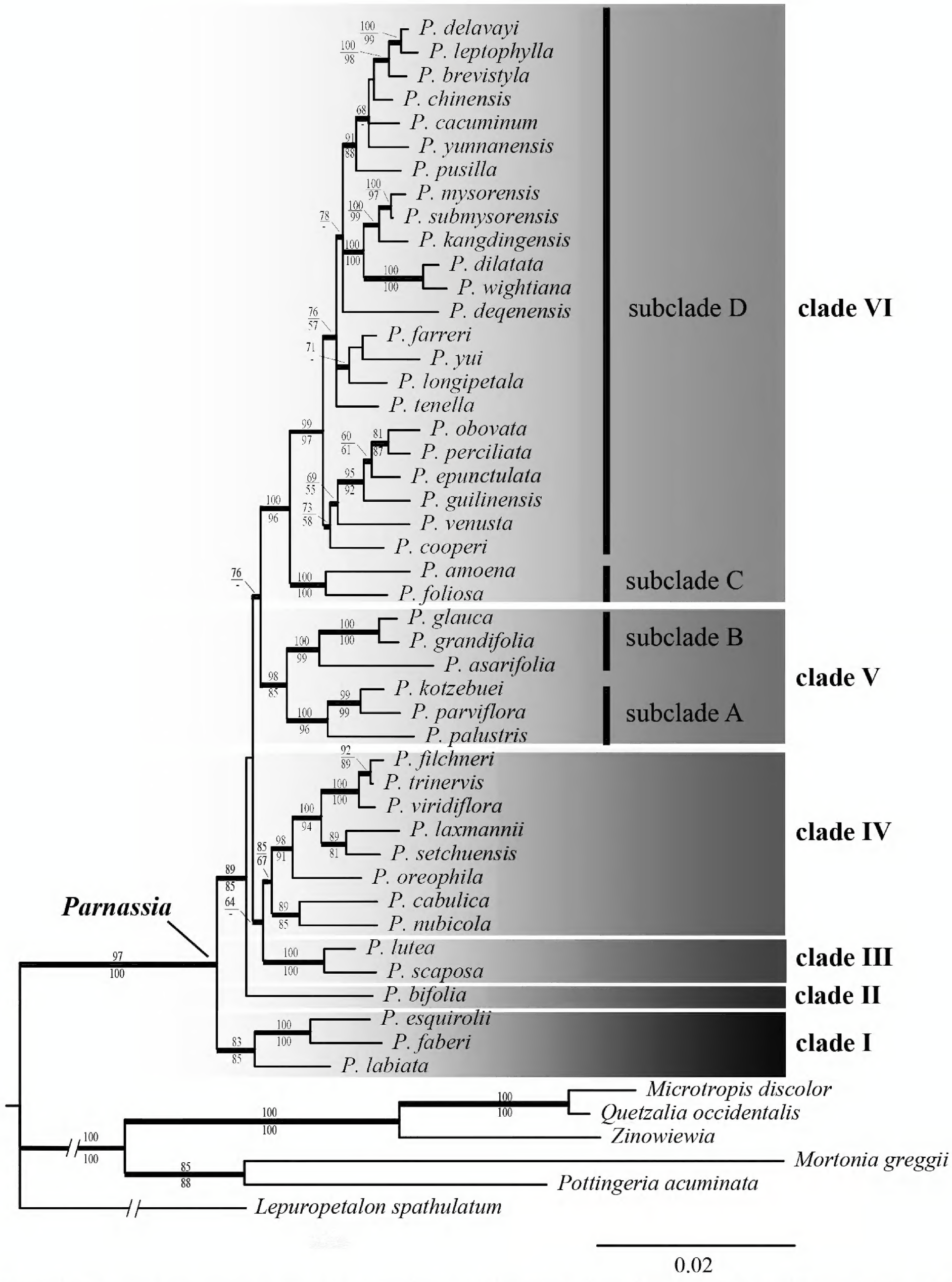

Figure 2. Fifty percent majority-rule consensus tree from the BI analysis of combined cpDNA data. Topologies from ML and MP analyses were congruent with the BI tree. Bayesian posterior probability values ≥ 0.90 are indicated by bold branches. MP/ML bootstrap values ≥ 50% are shown above/below branches, respectively, otherwise, a hyphen (-) is used. For *Zinowiewia*, combined sequences from multiple species are represented at the generic level.

this clade, *P. foliosa* and *P. amoena* formed a subclade sister to the remaining taxa. Despite limited resolution within clade VI, five of the six subclades were corroborated by unambiguous morphological synapomorphies.

Nuclear ribosomal ITS phylogeny

The nrDNA phylogeny employed *Lepuropetalon spathulatum* as the sole outgroup, accommodating high substitution rates in Celastraceae (Bai et al. 2015). MP analysis yielded multiple equally parsimonious trees differing mainly in clade placement (Fig. 3). Three species of *P.* sect. *Longiloba* Phillips (*P. asarifolia*, *P. grandifolia*, *P. glauca*) formed a highly supported clade (Clade VII, Fig. 3B), as did *P. palustris*, *P. kotzebuei*, and *P. parviflora* (Clade VI, Fig. 3B). Several clades showed complete concordance between plastid and nuclear topologies (e.g., Clade IX). Notably, *P.* sect. *Fimbripetalum* demonstrated strong monophyly (Clade VIII, Fig. 3B), while North American species formed a distinct clade, sister to *P. bifolia* (Clade II, Fig. 3B). The largest clade was well resolved but inconsistent support at internal nodes. *P. farreri* showed affinity to members of *P.* sect. *Saxifragastrum* (*P. longipetala*, *P. tenella*, *P. yunnanensis*), with which it shares staminodial morphology.

Six species from Drude's *P.* sect. *Nectarotrilobos* (equivalent to Ku's *P.* sect. *Nectarotrilobos* ser. *Nectarotrilobos*) formed a well-supported clade (BS=99, PP=1.0), sharing ovate-oblong basal leaves with truncate or cuneate bases. In particular, those species with simple three lobed staminodia, this similarity to Asian species may be the result of homoplastic evolution. The low sequence divergence within the Hengduan Mountains clade suggests either recent rapid ecological radiation or conservation of plesiomorphic floral traits, especially staminodial and petal morphology.

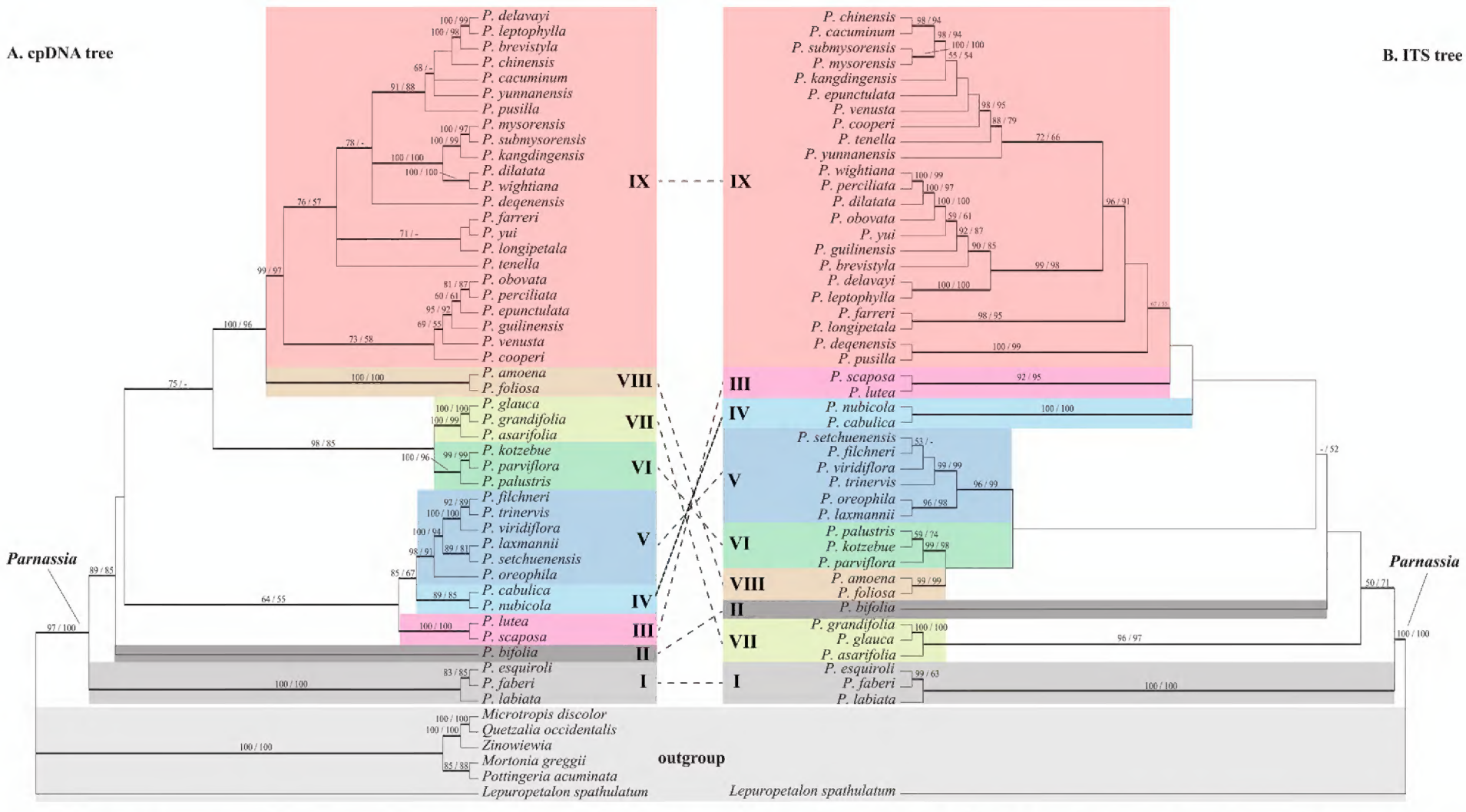

Figure 3. Phylogenetic relationships in *Parnassia* based on cpDNA (**A**) and nrDNA (**B**) data. Branch numbers indicate ML/MP bootstrap values. Dashed boxed highlight topological conflicts between ML trees. All branch lengths are significant (*P*=0.01).

Discussion

Reconciliation of molecular phylogeny with classification systems

The intricate taxonomic history of *Parnassia* largely stems from extreme morphological plasticity in staminodia, petal margins and floral pigmentation (e.g., Phillips 1982; Ku 1987; Wu et al. 2003; Wu 2005). Our cpDNA phylogeny identifies six well-supported clades, each definable by morphological or biogeographical features. Comparison with Ku's (1987) system reveals both concordance and conflict signals: (1) Engler's (1930) *P.* sect. *Cladoparnassia* and Ku's (1987) *P.* sect. *Saxifragastrum* ser. *Rosulatae* form monophyletic clade I; (2) Ku's (1987) monotypic *P.* sect. *Nectarobilobos* corresponds to clade II; (3) *P. lutea* and *P. scaposa* (Ku's *P.* sect. *Nectarotrilobos* subsect. *Amblysandra* ser. *Luteae*) comprise clade III; (4) a Drude's (1875) *P.* sect. *Nectarotrilobos* subgroup with subtle morphological variation (Handel-Mazzetti; Phillips 1982; Ku 1987) constitutes clade IV; (5) Phillips's (1982) sections, *P.* sect. *Parnassia* and *P.* sect. *Longiloba* form monophyletic clade V; and (6) the diverse clade VI amalgamates elements from Drude's (1875) sections *P.* sect *Saxifragastrum*, *P.* sect. *Fimbripetalum* and *P.* sect *Nectarotrilobos*, along with Ku's (1987) sections *P.* sect. *Odontohymen*, *P.* sect. *Allolobos*, and *P.* sect. *Nectaroquinquelobos*. These data illustrate the value of chloroplast gene sequence data in resolving genetic, and hence phylogenetic, relationships among members of the most taxonomically complex groups.

Members of each respective section share similar morphological traits or geographical distributions. Ku (1987) correctly recognized the affinity between bractless species *P. lutea* and *P. scaposa* (*P.* subsect. *Amblysandra* ser. *Luteae*). However, the limited molecular divergence within *P.* sect *Nectarotrilobos* – coupled with its remarkable morphological variation—indicates rapid radiation with extensive ecological diversification and speciation. The morphological diversity encompassed by this group is so great that subsequent workers have provided substantially modified concepts of relationships (Wu et al. 2003; Wu 2005). Because of the number of species representing many of the intermediates between extremes, it is not possible to adequately subdivide the section into smaller groups in a formal classification.

Evolutionary implications of morphological characters

Current *Parnassia* classification reflects traditional interpretation of evolutionary trends in the floral morphology, emphasizing staminodial elaboration and biogeography (Ku 1987). Our results, consistent with previous studies (Phillips 1982; Zhang and Simmons 2006), demonstrate that staminodial morphology—the primary character in traditional classifications—exhibits considerable homoplasy. The two groups in North America are shown here to be highly distinct. *Parnassia* sect. *Nectarotrilobos* was the most species rich section and represented the most diversified clade in the genus. *Parnassia* species in the Sino-Himalayan region, particularly the Hengduan Mountains (Ku 1987; Wu et al. 2003; Wu 2005; Wu et al. 2009), exhibit greater variability. Eastern Asia and North America represent two centers of diversity for this genus. These findings advance the taxonomy of *Parnassia* and provide a foundation for studying its evolutionary diversity.

Traditional infrageneric classifications by Drude (1875), based mainly on staminodium shape and petal margin, were not supported by molecular data.

Sections characterized by long-fimbriate petals (*P.* sect. *Fimbripetalum*), mallet-shaped staminodes (*P.* sect. *Saxifragastrum*), or prominently 3–7 lobed staminodes (*P.* sect. *Nectarotrilobos*) were not monophyletic. The close relationship between *P. faberi*, *P. esquirolii* and *P. labiata* is not reflected in their classification. The classification of the Asian species presents rather more serious difficulties. Several groups are relatively distinct. *P. foliosa* and *P. amoena*, readily recognized by the presence of 3 to 9 cauline leaves on the peduncle and elaborate fimbriate petals, are members of Drude's (1875) section *Fimbripetalum*. Although *P. noemiae* was not included in this analysis, it is apparent from the description that it also belongs in section *Fimbripetalum*, having character states very similar to *P. foliosa* and *P. amoena*. Phylogenetic results suggest that mallet-shaped staminodes present the ancestral condition, while lobed or globose- tipped staminodes forms evolved independently. Without substantial evidence regarding relative ancestral or derived character states, it is difficult to evaluate which is the most primitive section.

Several nodes—particularly within *P.* sect. *Nectarotrilobos*— exhibited low support (BS < 80%, PP < 0.95), likely reflecting recent rapid radiation in the Himalaya – Hengduan region, incomplete lineage sorting, and insufficient phylogenetic signal. Limited marker variation and ongoing gene flow or hybridization may further blur genetic resolution. We observed topological conflicts between plastid and nuclear datasets. For infrageneric revision, we prioritized the cpDNA phylogeny due to its higher nodal support and topological stability. Plastid genomes offer advantages for resolving deep relationships given their uniparental inheritance and low recombination rates. ITS conflicts likely reflect faster evolution, incomplete lineage sorting, or historical hybridization— exemplified by putative hybrid *P. yui*, where cpDNA indicates *P. farreri* and *P. longipetala* as maternal donors. While cpDNA provides a robust classification framework, we acknowledge these conflicts and emphasize the need for future phylogenomic studies.

Although molecular data do not support staminode-based classifications, these results suggest that incomplete lineage sorting, hybridization, or gene introgression events may have frequently occurred among sympatric species. For instance, fringed petal margins evolved independently in *P. foliosa* and *P. farreri*, reflecting similar pollinator-mediated selection. Taxonomic difficulties in *Parnassia* also stem from two distinct morphological issues. First, characters once considered diagnostic have proven unreliable upon broader sampling. The prolongation of the anther connective, for example, was traditionally used to distinguish *P. delavayi*, *P. brevistyla*, and *P. leptophylla* (Franchet 1897). However, Yu et al. (2018) demonstrated that this trait varies continuously among populations and formally treated the latter two names as synonyms of *P. delavayi*. Second, continuously varying quantitative traits have frequently been partitioned into multiple segregate species, as seen in the P. wittiana complex (including *P. gansuensis*, *P. yiliangensis*, and *P. monochorifolia*; Wu et al. 2004; Wu 2005; Wang et al. 2018).

For the putative hybrid P. yui, chloroplast data indicate *P. farreri* and *P. longipetala* as maternal donors, though paternal ancestry remains unresolved.

The most important contribution of this study is not the proposal of a modified classification, but rather the evidence for interspecific relationships that can guide further investigations within the genus. Also distinct is the group composed of *P. esquirolii*, *P. faberi* and *P. labiata*. This section, *P.* sect. *Cladoparnassia*, is distinguished by its small habit and flowers, and

simple undivided or slightly transversely notched, club-shaped staminodia. *Lepuropetalon spathulatum*, the sister genus to *Parnassia*, was used as the outgroup in the parsimony analysis. Morphological comparisons between *Lepuropetalon* and the *P. faberi* group reveal shared traits such as reduced stature and simplified floral architecture, yet their phylogenetic distance precludes direct inference of ancestral character states without formal ancestral state reconstruction (Simmons et al. 2001; Zhang and Simmons 2006). Future studies employing explicit character mapping and broader outgroup sampling are needed to rigorously assess whether rosette leaves represent a plesiomorphic condition in *Parnassia*. Furthermore, topological conflicts between the plastid and nuclear ITS phylogenies were observed in the relationships of certain taxa within Clades V and VI (e.g., the *P. palustris* complex and *P. farreri*), suggesting that these lineages may have experienced hybridization, introgression, or incomplete lineage sorting. It should be noted that the Sanger sequencing markers employed in this study provide insufficient informative sites to resolve recent rapid radiations in the Hengduan Mountains region, contributing to low nodal support within Clade VI. Future studies integrating phylogenomic data are needed to further clarify these relationships.

Revised infrageneric classification

The center of diversity for *Parnassia* is in central Asia, especially the Himalayan axis where the majority of the species occur. Our molecular results largely contradict Ku's (1987) sectional boundaries but support the foundational framework of Drude and Engler. We propose the following revised infrageneric classification of *Parnassia* reflecting phylogenetic relationships; this revised classification is primarily based on the phylogenetic relationships revealed by the cpDNA tree (Fig. 2):

1. [clade I] *Parnassia* sect. *Cladoparnassia* Engl. in Engl. u Prantl, Nat. Pflanzenfam. Aufl. 2, 18a: 182. 1930

= *Parnassia* sect. *Saxifragastrum* Drude ser. *Rosulatae* T.C.Ku in Bull. Bot. Res. North- East. For. Univ. 7 (1): 28. 1987. – Type: *P. esquirolii* Lévl.

Type. *P. faberi* Oliv.

Diagnosis. The typical characteristic of this clade is that the basal leaves are numerous, densely clustered in a rosette. The staminodes are structurally simple, usually unlobed at the apex or only bilobed in a lip-like form.

2. [clade II] *Parnassia* sect. *Nectarobilobos* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 46. 1987

Type. *P. bifolia* Nekr.

Diagnosis. This clade is monospecific, containing only *P. bifolia*, which is distinguished by having two cauline leaves and staminodes that are flat and bilobed at the apex.

3. [clade III] *Parnassia* sect. *Luteae* (T.C.Ku) D.Wu & D.Z.Li, stat. nov.
urn:lsid:ipni.org:names:77378964-1

≡ *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Amblysandra* (Franch.) T.C.Ku ser. *Luteae* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 31. 1987.

Type. *P. lutea* Batal.

Diagnosis. A prominent feature of this clade is the absence of cauline leaves. The basal leaves are ovate to oblong, with a truncate or cuneate-decurrent base. The staminodes are flat and trilobed.

4. [clade IV] *Parnassia* sect. *Longifolia* D.Wu & D.Z.Li, sect. nov.
urn:lsid:ipni.org:names:77378965-1

Type. *P. oreophila* Hance.

Diagnosis. The morphological characteristics of this new section include the presence of one cauline leaf. The basal leaves are similar in morphology to those of Clade III, being ovate to oblong with a truncate or decurrent base. The staminodes are also flat and trilobed.

5. [clade V] *Parnassia* sect. *Parnassia*

≡ *Parnassia* sect. *Nectarodroson* Drude in Linnaea 39: 301. 1875 = *Parnassia* sect. *Amblysandra* Franch. in Bull. Soc. Bot. France 44: 251. 1897.

Type. *P. palustris* L.

Diagnosis. The basal leaves are usually cordate or reniform, with a cordate base. The staminodes are deeply lobed, reaching half or more of their length.

5a. [subclade A] *Parnassia* sect. *Parnassia* subsect. *Parnassia*

Type. *P. palustris* L.

Diagnosis. The typical features include staminodes that are flat with as many as 5–30 lobes. The seed coat is often sac-like.

5b. [subclade B] *Parnassia* sect. *Parnassia* subsect. *Longiloba* D.Wu & D. Z.Li, subsect. nov.
urn:lsid:ipni.org:names:77378966-1

Type. *P. grandifolia* DC.

Diagnosis. The staminodes are branched (not flat), usually with three lobes (rarely 4–5). The petals are entire. There is typically one cauline leaf. *P.* sect. *Longiloba* was first recognized by Phillips (1982) but neither effectively nor validly published; it is validated here as a new subsection.

6. [clade VI] *Parnassia* sect. *Nectarotrilobos* Drude in Linnaea 39: 302. 1875

≡ *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Amblysandra* (Franch.) T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 31. 1987 ≡ *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Amblysandra* (Franch.) T.C.Ku ser. *Nectarotrilobos* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 32. 1987 = *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Amblysandra* (Franch.) T.C.Ku ser. *Lijiangenses* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 32. 1987.

Type. *P. mysorensis* Heyne ex Wight & Arn.

Diagnosis. The basal leaves are mainly cordate or reniform, with a cordate base. The staminodes are either unlobed or lobed to a depth not exceeding half their length. The seed coat is compact or slightly sac-like.

6a. [subclade C] *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Fimbripetalum* (Drude) D.Wu & D.Z.Li, stat. nov.

urn:lsid:ipni.org:names:77378968-1

≡ *Parnassia* sect. *Fimbripetalum* Drude in Linnaea 39: 302. 1875 ≡ *Parnassia* sect. *Fimbripetalum* Drude ser. *Fimbripetalum* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 51. 1987. – Type: *P. foliosa* Hook. f. et Thomson.

= *Parnassia* sect. *Fimbripetalum* Drude ser. *Amoenae* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 51. 1987.

Type. *P. amoena* Diels.

Diagnosis. The petal margins bear fringed hairs. The plants often have three or more cauline leaves. The staminodes are branched.

6b. [subclade D] *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Nectarotrilobos*

= *Parnassia* sect. *Saxifragastrum* Drude in Linnaea 39: 301. 1875 ≡ *Parnassia* sect. *Saxifragastrum* Drude ser. *Saxifragastrum* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 22. 1987. – Type: *P. tenella* Hook. f. et Thomson.

= *Parnassia* sect. *Odontohymen* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 29. 1987. – Type: *P. farreri* W.E.Evans

= *Parnassia* sect. *Allolobos* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 46. 1987. – Type: *P. wightiana* Wall. ex Wight & Arn.

= *Parnassia* sect. *Nectaroquinquelobos* T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 52. 1987. – Type: *P. perciliata* Diels

= *Parnassia* sect. *Xiphosandra* Franch. in Bull. Soc. Bot. France 44: 262. 1897 ≡ *Parnassia* sect. *Nectarotrilobos* Drude subsect. *Xiphosandra* (Franch.) T.C.Ku in Bull. Bot. Res., Harbin. 7 (1): 44. 1987. – Type: *P. delavayi* Franch.

Type. *P. mysorensis* Heyne ex Wight & Arn.

Diagnosis. This subclade exhibits high morphological diversity and encompasses the principal lineages of Clade VI, excluding Subclade C (characterized

by fringed petals and branched staminodes). Its core morphological variations are manifested in: staminodes that are club-shaped to columnar (corresponding to the former sect. *Saxifragastrum*), flat and trilobed (corresponding to the former sect. *Nectarotrilobos* s.str.), flat and pentalobed (corresponding to the former sect. *Allolobos*), or short-fan- shaped with apical teeth (corresponding to the former sect. *Odontohymen*). Additionally, lineages with significantly elongated stamen connectives (corresponding to the former sect. *Xiphosandra*) are also nested within it. Despite these pronounced morphological differences, particularly in staminode structure, phylogenetic analyses consistently recover these groups as a single, well-supported monophyletic clade.

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References

APG IV (2016) An update of the Angiosperm Phylogeny Group classification f or the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 181(1): 1–20. https://doi.org/10.1111/boj.12385

Bacon CD, Simmons MP, Archer RH, Zhao LC, Andriantiana J (2016) Biogeo graphy of the Malagasy Celastraceae: multiple independent origins followed by widespread dispersal of genera from Madagascar. Molecular Phylogenetics and Evolution 94: 365–382. https://doi.org/10.1016/j.ympev.2015.09.013

Bai MY, Li YR, Zhu QJ, Ye CC, Liu SR (2015) Preliminary study on phylogenesis of *Parnassia* L. based on ITS sequence. Journal of Dali University 14(12): 82–88. https://doi.org/10.3969/j.issn.1672-2345.2015.12.018

Bohm BA, Donevan LS, Bhat UG (1986) Flavonoids of some species of Bergenia, Francoa, Parnassia, and Lepuropetalon. Biochemical Systematics and Ecology 14: 75–77. https://doi.org/10.1016/0305-1978(86)90088-8

Bonnin I, Colas B, Holl A, Hendoux F, Destine B, Viards F (2002) Populatio n structure of an endangered species living in contrasted habitats: *Parnassia palustris* (Saxifragaceae). Molecular Ecology 11(5): 979–990. https://doi.org/10.1046/j.1365-294x.2002.01499.x

Borgen L, Hultgård UM (2003) *Parnassia palustris*: a genetically diverse species in Scandinavia. Botanical Journal of the Linnean Society 142(3): 347–372. https://doi.org/10.1046/j.1095-8339.2003.00186.x

Dai XS, Min DZ, Yang B, Wu D, Li B (2021) Taxonomic note of *Parnassia* (Celastraceae) in China II: population surveys reveal that *P. guilinensis* is conspecific to *P. xinganensis*. PhytoKeys 172: 67–73. https://doi.org/10.3897/phytokeys.172.62749ys.172.62749

Dai XS, Tian S, Wu W, Yang B, Ma YF, Ge G, Wu D (2023) Phylogeography of alpine plant *Parnassia*. Botany 101(9): 414–423. https://doi.org/10.1139/cjb-2023-0011b-2023-0011

Dandy JE (1927) The genera of saxifragaceae. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 1927(6): 100–118. https://doi.org/10.2307/4107540

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantiti es of fresh leaf material. Phytochemical Bulletin 19(1): 11–15.

Drude O (1875) Über die Blüthengestaltung und die Verwandtschaftsverhältnisse des Genus *Parnassia*, nebst einer systematischen Revision seiner Arten. Linnae a 39: 239–324. https://cir.nii.ac.jp/crid/1571417125714206976

Engler A (1930) Saxifragaceae. In: Engler A, Prantl K (Eds) Die Natürlichen Pflanzenfamilien (Ed. 2, Vol. 18a.) Verlag von Wilhelm Engelmann, Leipzig, 8–182. https://cir.nii.ac.jp/crid/1573668923909942272

Engler A (1964) In: Melchior H, Werdermann EG (Eds) Syllabus der Pflanzenf amilien, Ed. 12, Vol. 2. Gebrüder Borntraeger, Berlin, 137–138.

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). Kew Bulletin 52(1): 111–120. https://doi.org/10.2307/4117844

Ford CS, Ayres KL, Toomey N, Haider N, Van Alphen Stahl J, Kelly LJ, Wi kström N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MW, Wil kinson MJ (2009) Selection of candidate coding DNA barcoding regions for us e on land plants. Botanical Journal of the Linnean Society 159(1): 1–11. https://doi.org/10.1111/j.1095-8339.2008.00938.x

Franchet A (1897) Les *Parnassia* de l'Asie Orientale. Bulletin de la Société B otanique de France 44: 244–263. https://www.tandfonline.com/doi/pdf/10.1080/00378941.1897.10830772

Gornall RJ, Al-Shammary KIA (1998) Parnassiaceae. In: Cutler DF, Gregory M (Eds) Anatomy of the Dicotyledons: Saxifragales (Ed. 2, Vol. 4). Clarendon Press, Oxford, 245–247. https://doi.org/10.1093/oso/9780198547921.003.0025

Gu TC, Hultgård UM (2001) *Parnassia*. In: Wu ZY, Raven PH (Eds) Flora of China (Vol. 8) (Brassicaceae through Saxifragaceae). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 95–118.

Handel-Mazzetti H (1941) Die Chinesischen *Parnassia*-Arten. Oesterreichische Botanische Zeitschrift 90(2): 127–136. https://www.jstor.org/stable/23652889

Hooker JD, Thompson T (1858) Præcursores ad floram Indicam. Journal of the Proceedings of the Linnean Society, Botany 2: 54–103.

Hultgård UM (1987) *Parnassia palustris* L. in Scandinavia. Acta Universitatis Upsaliensis: Symbolae Botanicae Upsalienses 28(1): 1–128.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software v ersion 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kress WJ, Erickson DL (2007) A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. PLoS ONE 2(6): e508. https://doi.org/10.1371/journal.pone.0000508

Ku TC (1987) A revision of *Parnassia* (Saxifragaceae) in China. Bulletin of Botanical Research, Harbin 7(1): 1–59.

Ku TC (1995) Parnassioideae Engl. In: Lu L, Hwang S (Eds) Flora Reipublica e Popularis Sinicae (Vol. 35(1)). Science Press, Beijing, 66 pp.

Murbeck S (1918) Über die Organisation und verwandtschaftlichen Beziehungen der Gattung *Lepuropetalon*. Arkiv för Botanik 15(13): 1–12.

Nekrassova V (1917) *Parnassia*. In: Fedchenko BA (Ed.) Flora Aziatskoj Rossii 11. Pereselenskoe upravlenie Ministerstva zemledelija, Petrograd, 39 pp.

Phillips RB (1982) Systematics of *Parnassia* L. (Parnassiaceae): Generic overview and revision of North American taxa. PhD Thesis, University of California, Berkeley, USA.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Simmons MP, McKenna MJ, Bacon CD, Yakobson K, Cappa JJ, Archer RH, Ford AJ (2012) Phylogeny of Celastraceae tribe Euonymeae inferred from morphological characters and nuclear and plastid genes. Molecular Phylogenetics and Evolution 62: 9–20. https://doi.org/10.1016/j.ympev.2011.08.022

Shu YM (2017) A taxonomic revision of *Parnassia* L. (Celastraceae) from Pan-Himalaya. PhD Thesis, Beijing Forestry University, Beijing, China.

Shu YM, Yu HY, Mu XY, Zhang ZX (2017) Checklist and typification of *Parnassia* (Celastraceae). Phytotaxa 309(1): 1–22. https://doi.org/10.11646/phytotaxa.309.1.1

Shu YM, Zhang ZX (2017) Four new synonyms and a new combination in *Parnassia* (Celastraceae). PhytoKeys 77: 81–88. https://doi.org/10.3897/phytokeys.77.11513

Simmons MP (2004) Parnassiaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants (Vol. 6). Springer-Verlag, Berlin, 291–296. https://doi.org/10.1007/978-3-662-07257-8_33

Simmons MP, Savolainen V, Clevinger CC, Archer RH, Davis JI (2001) Phylogeny of the Celastraceae inferred from 26S nuclear ribosomal DNA, phytochrome B, *rbcL*, *atpB*, and morphology. Molecular Phylogenetics and Evolution 19(3): 353–366. https://doi.org/10.1006/mpev.2001.0937

Soltis DE, Soltis PS (1997) Phylogenetic relationships in Saxifragaceae sensu lato: a comparison of topologies based on 18S rDNA and *rbcL* sequences. American Journal of Botany 84(4): 504–522. https://doi.org/10.2307/2446027

Soltis S, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences of the United States of America 97: 7051–7057. https://doi.org/10.1073/pnas.97.13.7051

Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Xi Z, Rushworth CA, Gitzendanner MA, Sytsma KJ, Qiu Y-L, Hilu KW, Davis CC, Sanderson MJ, Beaman RS, Olmstead RG, Judd WS, Donoghue MJ, Soltis PS (2011) Angiosperm phylogeny: 17 genes, 640 taxa. American Journal of Botany 98: 704–730. https://doi.org/10.3732/ajb.1000404

Spongberg SA (1972) The genera of Saxifragaceae in the southeastern United States. Journal of the Arnold Arboretum 53(4): 409–498. https://doi.org/10.5962/p.324705

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and p ost-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Swofford DL (2002) PAUP. Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts. https://cir.nii.ac.jp/crid/1573950400678852992

Taberlet P, Pautou G, Gielly L, Bouvet J (1991) Universal primers for amplific ation of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17(5): 1105–1109. https://doi.org/10.1007/bf00037152

Thorne RF (1976) A phylogenetic classification of the angispermae. Evolutionary Biology 9: 35–106. https://doi.org/10.1007/978-1-4615-6950-3_2

Wang YP, Chen S, Shu YM, Li B, Wu D (2018) Taxonomic note of *Parnassia* (Celastraceae) in China: The identity of *P. wightiana*. Phytotaxa 375(1): 130–134. https://doi.org/10.11646/phytotaxa.375.1.10

Weber WA (2003) The middle Asian element in the southern Rocky Mountain flora of the western United States: A critical biogeographical review. Journal of Biogeography 30(5): 649–685. https://doi.org/10.1046/j.1365-2699.2003.00864.x

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencin g of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: A Guide to Methods and Appli cations. Academic Press, San Diego, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wu CY, Lu AM, Tang YC, Chen ZD, Li DZ (2003) The Families and Gener a of Angiosperms in China: A Comprehensive Analysis. Science Press, Beijing, 649–654.

Wu D (2005) Phylogeny and Biogeography of the genus *Parnassia*. PhD Thesis, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Wu D, Gao B, Wang H, Li D (2004) Four new synonyms of four species in the genus *Parnassia* (Parnassiaceae). Acta Botanica Yunnanica 26(6): 628–630.

Wu D, Gao LM, Moeller M (2009) Taxonomic notes on *Parnassia* section *Saxifragastrum* (Parnassiaceae) from China. Annales Botanici Fennici 46(6): 559–565. https://doi.org/10.5735/085.046.0609

Xia MZ, Li Y, Zhang FQ, Yu JY, Khan G, Chi XF, Xu H, Chen SL (2022) Reassessment of the phylogeny and systematics of Chinese *Parnassia* (Celastraceae): A thorough investigation using whole plastomes and nuclear ribosomal DNA. Frontiers in Plant Science 13: e855944. https://doi.org/10.3389/fpls.2022.855944

Yang JB, Wang YP, Möller M, Gao LM, Wu D (2012) Applying plant DNA barcodes to identify species of *Parnassia* (Parnassiaceae). Molecular Ecology R esources 12(2): 267–275. https://doi.org/10.1111/j.1755-0998.2011.03095.x

Yu HY, Guo FY, Shu YM, Zhang ZX (2018) Taxonomic note of *Parnassia* (Celastraceae) in China: A reassessment of Subsect. *Xiphosandra*. PhytoKeys 114: 43–54. https://doi.org/10.3897/phytokeys.114.30551

Zhang LB, Simmons MP (2006) Phylogeny and delimitation of the Celastrales inferred from nuclear and plastid genes. Systematic Botany 31(1): 122–137. htt ps://doi.org/10.1600/036364406775971778

Supplementary material 1

Voucher information and GenBank accession numbers for *Parnassia* and outgroup taxa sampled in this study

Authors: Wen-Xuan Zhang, Hong-Tao Li, Jin-Mei Lu, Ding Wu, De-Zhu Li

Data type: xlsx

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

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Author contributions

Wen-Xuan Zhang and Hong-Tao Li contributed equally to this work. They performed data analysis and drafted the manuscript. Jin-Mei Lu performed the experimental investigation and contributed to methodology. Ding Wu conceived and designed the study, performed experimental investigation, and contributed to writing and editing the manuscript. De-Zhu Li acquired funding, supervised the research, and critically reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

The newly generated nucleotide sequences (plastid and ITS) have been deposited in the GenBank database. All data supporting the findings of this study are available within the manuscript and its supplementary material.